CONCERNING THE CONSTANCY OF THE PROTEIN ELECTROPHORETIC PATTERN OF A POTATO TUBER VARIETY[1]

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Abstract

Tubers of a given variety from the same plant and from different plants grown in the same area and in diverse geographical locations were found to have visually indistinguishable protein patterns with acrylamide gel electrophoresis. Although total protein levels were considerably different for a given variety grown in two separate locations, their protein patterns were essentially similar. On storage four of the five varieties showed small but distinct alteration in the mutual density relationship of some of their bands. It is concluded that variety identification based on the protein patterns alone, particularly of stored tubers, might be ineffectual in those cases where the protein patterns lacked sufficient unique character.

A number of publications have appeared in the last few years dealing with the identification of potato varieties through their soluble protein patterns obtained by electrophoresis. Labib (5) and Zwartz (12) claimed that tuber varieties could be differentiated by measuring the relative heights of five of the six observed bands on paper electropherograms. This method was subsequently refined by Zwartz (13) to the area integration of nine stained bands on agar plate electropherograms. Loeschcke and Stegemann (7) differentiated 21 varieties by their qualitative protein patterns produced with acrylamide slab electrophoresis. Protsenko (10) also found that the electrophoretic pattern of potato tuber proteins on acrylamide gel differed with potato strains. Using a combination of electrophoretic protein patterns with those of peroxidase and esterase isoenzymes, Desborough and Peloquin (3, 4) were able to resolve the identity of 45 potato varieties. The basis of these identifications in all of the foregoing work rests upon a reproducible protein pattern that is wholly dependent on genetic and not ecological factors.

For several years at this laboratory we have used acrylamide gel electrophoresis to study the protein of a relatively small number of varieties. During this period we had not been fully convinced of the absolute reproducibility of the protein patterns. This, in combination with the nearly similar character of the patterns of some varieties and progeny of genetic crosses, had led us to question the extent that this method might serve for variety identification. With this in mind, we felt it important to carefully examine the protein patterns of individual tubers from a single plant and of different plants of a given variety grown in diverse geographical locations. Moreover, the effects of tuber storage and the stability of the protein preparations with time was also subjected to inquiry.

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Materials and Methods

Protein preparations were made of Norland, Katahdin, Red Pontiac, Kennebec, Russet Burbank and Irish Cobbler. The mature tubers of the first two varieties were obtained from a farm near Tripoli, Pennsylvania for those comparison studies of tubers originating from a single plant and plants from a single field. Katahdin tubers grown in New Jersey and Maine were obtained from Dr. Charles Cunningham at Campbell Institute, Riverton, New Jersey for comparison of their electrophoretic protein patterns with those from Pennsylvania.

Red Pontiac, Kennebec, Russet Burbank and Irish Cobbler samples grown in the Red River Valley were furnished by Mr. Roy Shaw of the Red River Valley Potato Processing Laboratory, East Grand Forks, Minnesota, and the same varieties, grown in Aroostook County, Maine were supplied by Dr. Hugh Murphy, University of Maine, Orono, Maine.

The potato protein preparations were made by grating the whole washed tuber on an inert plastic grater with the presence of sufficient $NaHSO_3$ to produce a 0.1% solution with the juice. The collected mash and juice were squeezed through a double layer of cheesecloth and the turbid solution centrifuged initially at 12,000 RCF for 30 min. at 2 C: the supernatant was recentrifuged at 31,700 RCF for 10 min. The grating and filtering operations were carried out in a 10 C room. The juice was not dialyzed nor subjected to permeation chromatography for removal of small molecules since earlier experiments had failed to demonstrate any resolution improvement or notable difference in the protein pattern if this were done prior to electrophoresis.

A 2.0 ml aliquot of the clear juice was made to 5% with respect to sucrose added just prior to sample application on the gel cylinder. Conditions of the gel preparation and electrophoresis were standardized and adhered to rigidly. The electrophoresis was carried out on 6% persulfate catalyzed resolving gel with a 2.5% riboflavin photocatalyzed stacking gel. The gels were cylindrical columns of 5 mm diameter and the distance of permissible migration maintained as constant as possible at 68 mm. A slightly modified (Buchler Instruments Company manual) tris-glycine buffer system of Davis (1) was employed. The electrophoresis was carried out at 10 C with a current of 4 mA per gel and the protein stain was 1% amido black in 7.5% acetic acid. A flowing, constantly renewed 7.5% acetic acid bath was used for destaining.

Whenever possible, samples for comparison were run simultaneously and the stained gels were visually examined directly. Photographs were prepared for future reference. In addition, line drawings were prepared in which the strength and width of the bands were indicated. Each protein band was assigned a letter which identifies its presence in each variety.

Earlier efforts to use the Joyce-Loebl densitometer for quantitative evaluation of the protein bands on the cylindrical acrylamide gels proved very unsatisfactory, largely because of the uneven background of the destained gel and close proximity of the bands. A comparative visual examination of the mutual relationshihp of the bands followed by careful photographic and line drawing representation proved more satisfactory.

In an effort to ascertain the reliability of protein preparations vs. their storage time, protein extracts of (Pa.) Katahdin tubers from a single

plant were made at intervals extending over a period of 7 days. Each new preparation was immediately electrophoresed and all prior preparations were re-electrophoresed along with it.

Red Pontiac, Russet Burbank, Kennebec and Irish Cobbler tubers were stored for approximately five months at 10 C, and the variety Katahdin for 57 days at 10 C. The protein fractions were prepared and electrophoresed as described above and compared with those of freshly harvested tubers.

Total soluble protein was determined by the biuret method (6) following 10% trichloroacetic acid precipitation and washing with ethanol.

Results

In the extract-time study, the protein bands obtained for variety Katahdin on preparations as old as seven days were indistinguishable from those immediately prepared. Although no change in the protein pattern was observed some flocculation or precipitation of protein had apparently begun after four days which only became considerable after prolonged storage (ten or more days).

The varieties Norland and Katahdin clearly have visually distinguishable protein patterns (Fig. 1). Protein patterns of all Norland tubers studied were visually identical. Twenty-four protein bands were discernible.

Protein patterns of all the Katahdin tubers studied were identical. As in the case of the Norlands, both the qualitative protein species and the relative density relationship between the bands were visually the same no matter whether the tubers of a given variety were obtained from the same plant, different plants in the same field or from entirely separate locations. Twenty bands were discerned in the Katahdin pattern.

The protein patterns of Red Pontiac, Russet Burbank, Kennebec and Irish Cobbler grown in Maine were similar to those from the Red River Valley. One difference observed, however, was that the "G" band (Fig. 2) was noticeably weaker in the Red River Valley grown Red Pontiacs than in the corresponding band found in that variety from Maine. Although the protein patterns of the four varieties grown in two separate locations were essentially alike, the total soluble protein content was markedly different for each variety (Table 1). If expressed on a dry weight basis the slightly greater solids content of the Red River Valley grown tubers would only have a small effect on the dissimilar protein values presented in Table 1.

The effects of tuber storage on the protein pattern was not consistent. The Katahdin tubers stored for 57 days were considerably desiccated and well sprouted, yet the protein pattern (Fig. 2) did not reveal any alterations from that of the prestored tubers. On the other hand, the other varieties showed some noticeable changes in their protein pattern after storage. An increase in the "D_1 D_2" bands was most apparent in the Russet Burbank pattern. The Red Pontiac tubers showed a possible reduction on the "F" band and an increase in the "G" band while Irish Cobbler and Kennebec demonstrated a marked decrease in "G" and, in the case of the Kennebecs, of the "H" band. There was also a suggested increase of "D_2" on storage of Irish Cobbler.

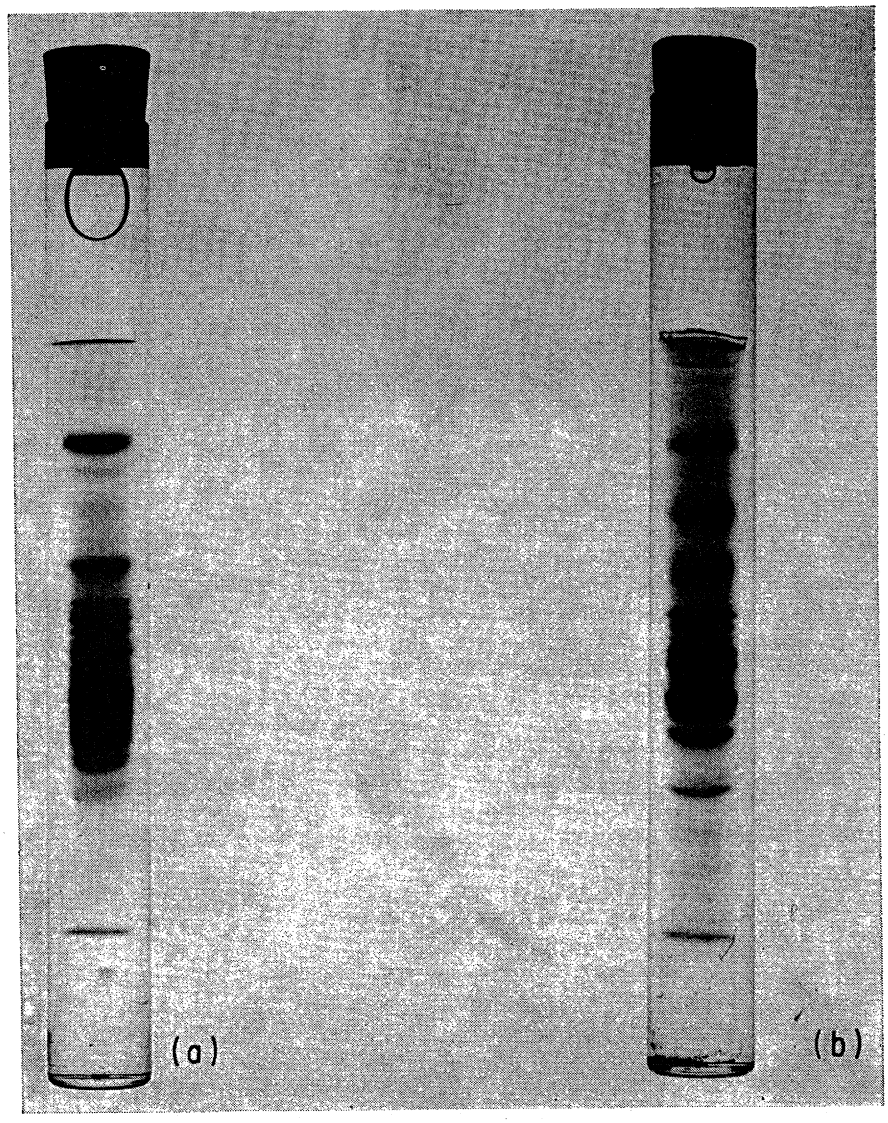

FIG. 1.—Protein electrophoretic patterns.
a. Variety Norland b. Variety Katahdin

DISCUSSION

Essentially the findings of this study are in agreement with those of the earlier workers (3, 4, 5, 7, 14). Each variety appears to have a persistent and somewhat unique electrophoretic protein pattern which is largely unaffected by geographical area of growth. The observed variation

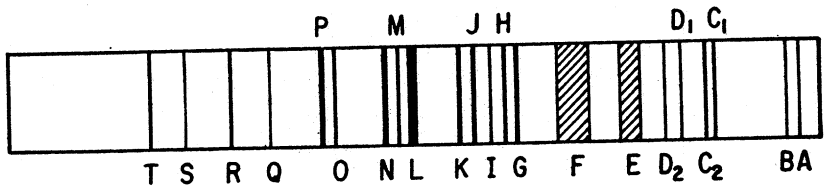

FIG. 2.—Diagrammatic representation of protein pattern of variety Katahdin showing band letter assignment.

TABLE 1.—*Total soluble protein content of four potato tuber varieties grown at two locations.*

Variety	mgs. soluble protein/g. fresh weight of tuber	
	Red River Valley	Aroostook, Maine
Red Pontiac	7.10	5.19
Kennebec	7.86	5.71
Irish Cobbler	8.26	5.14
Russet Burbank	7.12	4.29

in the intensity of the "G" band between Red River Valley and Maine grown varieties is one of the few fluctuations in the patterns of those examined in this study. It also represents one of the few bands exhibiting some alteration during tuber storage.

In the comparative study of tubers obtained from the same plant, care had been taken to choose those which presumably had set at the same period in growth as judged by tuber size. However, patterns provided by smaller but mature tubers from a later setting of the plant were visually identical.

Zwartz (14) found no differences in the electropherograms between the protein patterns of tubers that were greenhouse grown compared to those field grown although the protein content of the former was greater than the latter. Loeschcke and Stegemann (7) also reported a similar finding between some varieties grown in different regions. Zwartz, reporting slight differences in the electrophoretic pattern of some varieties grown in two clearly different soil types, minimized the observation since it mainly involved the slowest and fastest moving fractions. Regarding fertilization Zwartz comments that Labib's electropherograms (5) do not support his conclusions that there are no protein pattern differences. On the other hand, she reports that not even divergent nitrogen fertilization produced dissimilar protein patterns with a given variety. Our interpretation of the author's published electropherograms would not quite concede that point. The mutual relationship of some of the bands appears to have been altered. Loeschcke and Stegemann (7) do not seem as certain of the effects of diverse conditions of fertilization. The question might well

be asked, does an increase in protein for a given variety, resulting from some agricultural practice, simply reflect an increase in all of the protein species of the pattern? It would seem that certain environmental factors should affect the metabolically active components, in particular the enzyme levels if not the storage and structural proteins. Variety characterization, which is dependent on the peroxidase and esterase isoenzyme pattern, had not been studied in relation to storage and ecological effects (3, 4). Peroxidase isoenzymes from tobacco culture medium have been shown to change with culture age and growth temperature (9), and the peroxidase isoenzyme pattern of roots of pea seedlings grown in a high saline medium has been altered (11). Loeschcke and Stegemann (7) and work in this laboratory (12) have shown that detectable protein bands may or may not correspond with those stained for isoenzymes. In fact such correspondence, which often purports correlation, need not be the case at all. From electropherograms alone it would be rather difficult to conclude that a superimposed protein staining band and one responding to an enzyme substrate are identical. Moreover, it is difficult to ascertain if there is really any alteration in the mutual relationship of protein staining band patterns as affected by ecological factors when employing those methods described by the investigators discussed herein. Only relatively large changes would be detected and those reflecting most enzymes would not be discerned with a general protein stain because of its limited sensitivity. Many of the isoenzyme reactions have appeared on the acrylamide gels in a location devoid of a protein staining band. Nye et al. (8) described a lack of correlation between some bands showing polyphenol oxidase activity and a soluble protein band.

Loeschcke and Stegemann found that the mutual intensity relationship of the protein components remain remarkably constant on storage of 3 to 7 months after harvest and permit variety identification. Desborough and Peloquin had similar findings (2) and Zwartz (14) found only slight changes associated with storage until the tuber formed enormous sprouts whereby it was concluded that for sprout formation the soluble proteins are either not primarily used or are utilized at exactly equal rates.

The results of the current storage study are not consistent. The badly desiccated, sprouted Katahdin tuber presented a nearly identical pattern with a freshly harvested one. On the other hand, the other four varieties after somewhat longer storage than the Katahdin showed noticeable changes were possible in the "D_1 D_2", "F" and "G" bands of their patterns. In addition, the change need not be in the same direction, e.g. the "G" band. Conceivably, these alterable bands represent modifying enzymatic proteins in greater preponderance within these four varieties.

Perhaps, in most cases, there is sufficient uniqueness in the variety's protein pattern that relatively small alterations would not impair variety identification. However, in this laboratory, patterns have been observed so similar in character that small modifications in the protein staining bands occasioned by inconsistencies in the electrophoretic method, or by storage or ecological factors might render this method ineffectual. This will be noted in greater detail in a further publication (12).

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